AU 9647584 A 960807 9646
Priority Data (CC No Date): US 376327 (950120)
Applications (CC,No,Date): AU 9647584 (960119); WO 96US596 (960119)
Abstract (Basic): WO 9622362 A

Purified prepn. of primate embryonic stem cells (ESC) has the following properties: (a) can proliferate in vitro for over a year; (b) maintain the normal karyotype, and retain ability to differentiate to derivs. of endoderm, mesoderm and ectoderm, throughout long-term culture; and (c) will not differentiate on a fibroblast feeder layer.

USE - The cells are used to generate transgenic primates as models of specific human genetic diseases where the gene responsible has been cloned, and in tissue transplants by adjusting culture conditions to generate specific cell types (blood, neurological or muscle cells), or by allowing the cells to differentiate in tumours. The differentiated cells can also be isolated, and transplanted to treat haematopoietic, endocrine or degenerative neurological disease or hair loss, e.g. Parkinson's disease, juvenile onset diabetes or AIDS.

ADVANTAGE - The cells resemble human cells, and can be kept in the undifferentiated state, while remaining euploid, for long periods.

Dwg.0/6

25/3,AB/19 (Item 1 from file: 352)
DIALOG(R)File 352:DERWENT WPI

010857566 WPI Acc No: 96-354519/35
XRAM Acc No: C96-111747

Purified primate embryonic stem cells capable of long term culture - for producing transgenic primates as models of human disease, and for prepn. of tissue transplants

Patent Assignee: (WISC) WISCONSIN ALUMNI RES FOUND
Author (Inventor): THOMSON J A
Patent Family:

CC Number	Kind	Date	Week	
WO 9622362	A1	960725	9635	(Basic)
AU 9647584	A	960807	9646	

Priority Data (CC No Date): US 376327 (950120)
Applications (CC,No,Date): AU 9647584 (960119); WO 96US596 (960119)
Abstract (Basic): WO 9622362 A

Purified prepn. of primate embryonic stem cells (ESC) has the following properties: (a) can proliferate in vitro for over a year; (b) maintain the normal karyotype, and retain ability to differentiate to derivs. of endoderm, mesoderm and ectoderm, throughout long-term culture; and (c) will not differentiate on a fibroblast feeder layer.

USE - The cells are used to generate transgenic primates as models of specific human genetic diseases where the gene responsible has been cloned, and in tissue transplants by adjusting culture conditions to generate specific cell types (blood, neurological or muscle cells), or by allowing the cells to differentiate in tumours. The differentiated cells can also be isolated, and transplanted to treat haematopoietic, endocrine or degenerative neurological disease or hair loss, e.g. Parkinson's disease, juvenile onset diabetes or AIDS.

ADVANTAGE - The cells resemble human cells, and can be kept in the undifferentiated state, while remaining euploid, for long periods.

Dwg.0/6

?

Set	Items	Description
S1	120719	STEM(W)CELL?
S2	9692	EMBRYONIC(W)S1
S3	2116211	CULTURE
S4	431009	FIBROBLAST?
S5	362	S2 AND S3 AND S4
S6	65547	STEM(W)CELL
S7	1820	EMBRYONIC(W)S6
S8	2116211	CULTURE
S9	431009	FIBROBLAST?
S10	68479	PRIMATE
S11	17948196	HUMAN
S12	281071	MONKEY OR SIMIAN
S13	5	S5 AND S10
S14	117	S5 AND S11
S15	9	S5 AND S12
S16	121	S13 OR S14 OR S15
S17	101	RD (unique items)
S18	13	NORMAL(W)KAROTYPE
S19	3801	NORMAL(W)KARYOTYPE
S20	3813	S18 OR S19
S21	4	S17 AND S20
S22	1013275	DIFFERENTIAT?
S23	61	S17 AND S22
S24	35948	ENDODERM OR MESODERM OR ECTODERM
S25	19	S23 AND S24

?t s23/3,ab/2,7,10,11,13,25,28,31,32,55,59,60,61

>>>No matching display code(s) found in file(s): 12, 43, 129-130, 140, 158, 187, 189, 376, 428-429, 446, 449, 452, 455-456, 636

23/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08407238 95372375

Isolation of a primate embryonic stem cell line.

Thomson JA; Kalishman J; Golos TG; Durning M; Harris CP; Becker RA; Hearn JP

Wisconsin Regional Primate Research Center, University of Wisconsin, Madison 53715-1299, USA.

Proc Natl Acad Sci U S A (UNITED STATES) Aug 15 1995, 92 (17) p7844-8, ISSN 0027-8424 Journal Code: PV3

Contract/Grant No.: RR-00167, RR, NCRR; HD26458, HD, NICHD

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Embryonic stem cells have the ability to remain undifferentiated and proliferate indefinitely in vitro while maintaining the potential to differentiate into derivatives of all three embryonic germ layers. Here we report the derivation of a cloned cell line (R278.5) from a rhesus monkey blastocyst that remains undifferentiated in continuous passage for > 1 year, maintains a normal XY karyotype, and expresses the cell surface markers (alkaline phosphatase, stage-specific embryonic antigen 3, stage-specific embryonic antigen 4, TRA-1-60, and TRA-1-81) that are characteristic of human embryonal carcinoma cells. R278.5 cells remain undifferentiated when grown on mouse embryonic fibroblast feeder layers but differentiate or die in the absence of fibroblasts, despite the presence of

recombinant human leukemia inhibitory factor. R278.5 cells allowed to differentiate in vitro secrete bioactive chorionic gonadotropin into the medium, express chorionic gonadotropin alpha- and beta-subunit mRNAs, and express alpha-fetoprotein mRNA, indicating trophoblast and endoderm differentiation. When injected into severe combined immunodeficient mice, R278.5 cells consistently differentiate into derivatives of all three embryonic germ layers. These results define R278.5 cells as an embryonic stem cell line, to our knowledge, the first to be derived from any primate species.

23/3,AB/7 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13192364 BIOSIS Number: 99192364
Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts
Thomson J A; Kalishman J; Golos T G; Durning M; Harris C P; Hearn J P
Wisconsin Regional Primate Research Center, Univ. Wisconsin, 1223 Capitol Court, Madison, WI 53715-1299, USA
Biology of Reproduction 55 (2). 1996. 254-259.
Full Journal Title: Biology of Reproduction
ISSN: 0006-3363
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 008 Ref. 124371
We report the derivation of eight pluripotent cell lines from common marmoset (Callithrix jacchus) blastocysts. These cell lines are positive for a series of markers (alkaline phosphatase, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81) that characterize undifferentiated human embryonal carcinoma cells and rhesus embryonic stem cells. All eight cell lines had a modal chromosome number of 46; seven cell lines were XX and one was XY. Two cell lines (Cj11 and Cj62) were cultured continuously for over a year and remained undifferentiated and euploid. In the absence of fibroblast feeder layers, these cell lines differentiated to multiple cell types, even in the presence of leukemia inhibiting factor. Differentiated cells secreted bioactive CG into the culture medium and expressed alpha-CG, beta-CG, and alpha-fetoprotein mRNA, indicating trophoblast and endoderm differentiation. Bioactive CG secretion in differentiating cells was increased substantially in the presence of GnRH agoniSt D-Trp-6-Pro-9-NHEt. When grown at high densities, these cells formed embryoid bodies with a close resemblance to early postimplantation embryos, including the formation of a yolk sac, amnion, and an embryonic disc with an early primitive streak. These results make these pluripotent cells strong candidates for marmoset embryonic stem cells.

23/3,AB/10 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

8599092 EMBASE No: 92275000
Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture
Matsui Y.; Zsebo K.; Hogan B.L.M.
Department of Cell Biology, Tuberculosis/Cancer Research Inst., Sendai 980 Japan
CELL (USA) , 1992, 70/5 (841-847) CODEN: CELLB ISSN: 0092-8674

LANGUAGES: English SUMMARY LANGUAGES: English

Steel factor (SF) and LIF (leukemia inhibitory factor) synergistically promote the proliferation and survival of mouse primordial germ cells (PGCs), but only for a limited time period in culture. We show here that addition of bFGF to cultures in the presence of membrane-associated SF and LIF enhances the growth of PGCs and allows their continued proliferation beyond the time when they normally stop dividing in vivo. They form colonies of densely packed, alkaline phosphatase-positive, SSEA-1-positive cells resembling undifferentiated embryonic stem (ES) cells in morphology. These cultures can be maintained on feeder layers for at least 20 passages, and under appropriate conditions give rise to embryoid bodies and to multiple differentiated cell phenotypes in monolayer culture and in tumors in nude mice. PGC-derived ES cells can also contribute to chimeras when injected into host blastocysts. The long-term culture of PGCs and their reprogramming to pluripotential ES cells has important implications for germ cell biology and the induction of teratocarcinomas.

23/3,AB/11 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE

8459000 EMBASE No: 92135005
Embryonic stem cells as a model for cardiogenesis
Robbins J.; Doetschman T.; Jones W.K.; Sanchez A.
USA
TRENDS CARDIOVASC. MED. (USA) , 1992, 2/2 (44-50) CODEN: TCMDE ISSN: 1050-1738
LANGUAGES: English SUMMARY LANGUAGES: English

Embryonic stem (ES) cells are derived from the inner cell mass of mouse blastocysts. These cells, when placed upon a suitable fibroblast feeder layer, continue to proliferate without overt differentiation and remain totipotent. Cells in this state are competent for gene targeting via homologous recombination. Hence, they hold the possibility of developing defined animal models of human cardiovascular disease. When removed from the feeder layer, ES cells undergo differentiation and development into large, multicellular structures, termed embryoid bodies (EBs). Morphologic, biochemical, and molecular genetic analyses indicate that during EB development some early aspects of cardiogenesis are recapitulated. Thus, EB development in culture is useful for studying certain early cardiogenic events.

23/3,AB/13 (Item 1 from file: 94)
DIALOG(R)File 94:JICST-EPlus

01326683 JICST ACCESSION NUMBER: 91A0341243 FILE SEGMENT: JICST-E
Embryonic stem cell.
MIKI KIYOSHI (1); HANAOKA KAZUNORI (1)
(1) National Center of Neurology and Psychiatry
Shinkei Kenkyu no Shinpo(Advances in Neurological Sciences), 1991,
VOL.35,NO.1, PAGE.17-25, FIG.5, REF.41
JOURNAL NUMBER: Z0693AAP ISSN NO: 0001-8724
UNIVERSAL DECIMAL CLASSIFICATION: 591.3.05 575.113:575.23 57.082
LANGUAGE: Japanese COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Journal
ARTICLE TYPE: Review article

MEDIA TYPE: Printed Publication
ABSTRACT: Pluripotent embryonic stem (ES) cells established from normal mouse embryos provide promising experimental system to analyze the function of genes underlying the development of mammals and produce model animals for the hereditary human disease. ES cells continue to grow as undifferentiated stem cells so far as they are cultured on feeder cells of mitomycin C treated STO fibroblasts. Altering the growth environment of the cell, for example, cultivating the cell in suspension on bacteriological petri dishes or transplanting the cell into an adult syngeneic mouse subcutaneously forces the cell to differentiate into a variety of cell types in vitro or in vivo. When the ES cells are microinjected into host blastocysts and subsequently incubated in a foster mother, they fully exhibit their developmental potency and differentiate into virtually all cell types in adult chimeras, and in some individuals they form functional gameres. This remarkable feature of ES cells allow them to serve as vehicles for manipulating mouse genomes: ES cells are subjected to introduction of a cloned gene in vitro, and then successfully mutated ES cells are selected, and finally chimeric mice are produced using the transformed cells. Recently, various strategies have been developed for introducing mutation in a desired genetic locus of ES cells by means of gene targeting: homologous recombination between an introduced mutated gene and an endogenous chromosomal allele. Combining the method of gene targeting and of making germ line chimeras using ES cells, it is now possible to generate genetically designed mouse strains heterozygous for the altered gene. Such mice could be inter-bred to produce offsprings homozygous for the altered gene. Thus, it is now becoming a reality to alter any desired genes in mouse genome by means of this experimental system. (abridged author abst.)

23/3,AB/25 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

162157 DBA Accession No.: 94-04708 PATENT
Maintaining in vitro animal embryonic stem cells without differentiation - in vitro human and animal embryonic stem cell culture in the presence of fowl embryo recombinant fibroblast feeder layer, or fowl embryonic stem cell-derived cell factor
PATENT ASSIGNEE: CSIRO; Cancer-Res.Campaign-Technol. 1994
PATENT NUMBER: WO 9403585 PATENT DATE: 940217 WPI ACCESSION NO.: 94-065675 (9408)
PRIORITY APPLIC. NO.: AU 923935 APPLIC. DATE: 920804
NATIONAL APPLIC. NO.: WO 93AU399 APPLIC. DATE: 930804
LANGUAGE: English
ABSTRACT: A method for maintaining in vitro animal embryonic stem (ES) cells (I) without substantial differentiation comprises culturing (I) in the presence of a feeder layer (II) comprising fowl embryonic fibroblasts (III). Also claimed are: i. a method as above except that (I) are cultured in the presence of fowl-derived ES cell factor; ii. an isolated fowl-derived ES stem cell factor comprising a protein of mol.wt. 20,000-30,000, an amino acid sequence in the N-terminal region of Xaa1-Pro-Val-Ala-Gly-Tyr-Xaa2, where Xaa1 = 4 unknown N-terminal amino acids, and Xaa2 = the remaining amino acids of the polypeptide, and is derived from (III) and from fowl embryo extracts; and iii. a non-human animal derived from ES cells cultured in the presence of a feeder layer comprising (III). The fowl embryonic fibroblast layer is a

confluent monolayer comprising fibroblastoid cells. (III) are first mutagenized by a chemical agent, UV irradiation or genetic manipulation prior to forming the confluent layer. This method enables the maintenance of human or animal primordial germ cells and hematopoietic stem cells. (51pp)

23/3,AB/28 (Item 3 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

14280792 Genuine Article#: TA938 Number of References: 48
Title: ISOLATION AND CHARACTERIZATION OF A FEEDER-DEPENDENT, PORCINE TROPHECTODERM CELL-LINE OBTAINED FROM A 9-DAY BLASTOCYST
Author(s): FLECHON JE; LAURIE S; NOTARIANNI E
Corporate Source: UNIV NEWCASTLE UPON TYNE,SCH MED,DEPT PHYSIOL SCI/NEWCASTLE TYNE NE2 4HH/TYNE & WEAR/ENGLAND/; BABRAHAM INST/CAMBRIDGE CB2 4AT//ENGLAND/; INRA,BIOL CELLULAIRE & MOLEC LAB/F-78352 JOUY EN JOSAS//FRANCE/
Journal: PLACENTA, 1995, V16, N7 (OCT), P643-658
ISSN: 0143-4004
Language: ENGLISH Document Type: ARTICLE
Abstract: We have established in culture a feeder-dependent cell line, termed TE1, from a 9 day pre-implantation, porcine embryo. TE1 cells were observed by light and electron microscopy, and characterized by immunocytochemistry: the morphology, cytology and ultrastructure of this cell line are described. The cells display epithelial characteristics, as revealed using immunofluorescence microscopy with antibody against cytokeratins of simple epithelia, but not with antibody against vimentin. The cells demonstrate many morphological and cytochemical features in common with trophectoderm of the intact porcine blastocyst. For example, TE1 cells are polarized and possess tight junctions at their borders, similar to those found in trophectoderm of the pre-implantation embryo. Moreover, TE1 cells label positively for the porcine trophectoderm-specific monoclonal antibody, SN1/38. Thus, by several important criteria TE1 is deduced to be a porcine trophectoderm cell line.

23/3,AB/31 (Item 6 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13790200 Genuine Article#: QQ271 Number of References: 75
Title: ISOLATION AND CHARACTERIZATION OF PERMANENT CELL-LINES FROM INNER CELL MASS CELLS OF BOVINE BLASTOCYSTS
Author(s): VANSTEKELENBURGHAMERS AEP; VANACHTERBERG TAE; REBEL HG; FLECHON JE; CAMPBELL KHS; WEIMA SM; MUMMERY CL
Corporate Source: NETHERLANDS INST DEV BIOL,HUBRECHT LAB,UPPSALALAAN 8/3584 CT UTRECHT//NETHERLANDS/; NETHERLANDS INST DEV BIOL,HUBRECHT LAB/3584 CT UTRECHT//NETHERLANDS/; INRA/JOUY EN JOSAS//FRANCE/; AFRC,ROSLIN INST/ROSLIN/MIDLOTHIAN/SCOTLAND/; FREE UNIV AMSTERDAM HOSP,IVF LAB/AMSTERDAM//NETHERLANDS/
Journal: MOLECULAR REPRODUCTION AND DEVELOPMENT, 1995, V40, N4 (APR), P 444-454
ISSN: 1040-452X
Language: ENGLISH Document Type: ARTICLE
Abstract: Inner cell masses (ICM) from in vitro produced day 8 or 9 bovine

blastocysts were isolated by immunosurgery and cultured under different conditions in order to establish which of two feeder cell types and culture media were most efficient in supporting attachment and outgrowth of the bovine ICM cells. The efficiency of attachment and outgrowth of the ICM cells could be markedly improved when STO feeder cells were used instead of bovine uterus epithelial cells, and by using charcoal-stripped serum instead of normal serum to supplement the culture medium. More than 20 stable cell lines were obtained. Some of these lines were examined by immunofluorescence for developmentally regulated markers. From these results we conclude that the cell lines resemble epithelial cells, rather than pluripotent ICM cells. The developmental potential of cells of one of the lines was tested in the nuclear transfer assay. The cell line could support the initial development of enucleated oocytes, but none of the reconstructed embryos passed the eight-cell block. (C) 1995 Wiley-Liss, Inc.

23/3,AB/32 (Item 7 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13513739 Genuine Article#: PU209 Number of References: 37
Title: ISOLATION AND CULTURE OF INNER CELL MASS CELLS FROM HUMAN BLASTOCYSTS
Author(s): BONGSO A; FONG CY; NG SC; RATNAM S
Corporate Source: NATL UNIV SINGAPORE HOSP,DEPT OBSTET & GYNAECOL,LOWER KENT RIDGE RD/SINGAPORE 0511//SINGAPORE/
Journal: HUMAN REPRODUCTION, 1994, V9, N11 (NOV), P2110-2117
ISSN: 0268-1161
Language: ENGLISH Document Type: ARTICLE
Abstract: Totipotent non-committed inner cell mass (ICM) cells from human blastocysts, if demonstrated to be capable of proliferating in vitro without differentiation, will have several beneficial uses, not only in the treatment of neurodegenerative and genetic disorders, but also as a model in studying the events involved in embryogenesis and genomic manipulation. Nine patients admitted to an in-vitro fertilization programme donated 21 spare embryos for this study. All 21 embryos were grown from the 2-pronuclear until blastocyst stages on a human tubal epithelial monolayer in commercial Earle's medium (Medicult, Denmark) supplemented with 10% human serum. The medium was changed after blastocyst formation to Chang's medium supplemented with 1000 units/ml of human leukaemia inhibitory factor (HLIF) and the embryos left undisturbed for 72 h to allow the hatched ICM and trophoblast to attach to the feeder monolayer. Nineteen of the 21 embryos from nine patients produced healthy ICM lumps which could be separated and grown in vitro. Two of the lumps differentiated into fibroblasts while the remaining 17 (eight patients) produced cells with typical stem cell-like morphology, were alkaline phosphatase positive and could be maintained for two passages. It was possible to retain the stem cell-like morphology, alkaline phosphatase positiveness and normal karyotype through the two passages in all of them using repeated doses of HLIF every 48 to 72 h. This is the first report on the successful isolation of human ICM cells and their continued culture for at least two passages in vitro.

23/3,AB/55 (Item 30 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

12030578 Genuine Article#: KF500 Number of References: 22
Title: CRITERIA THAT OPTIMIZE THE POTENTIAL OF MURINE EMBRYONIC STEM-CELLS FOR INVITRO AND INVIVO DEVELOPMENTAL STUDIES
Author(s): BROWN DG; WILLINGTON MA; FINDLAY I; MUGGLETONHARRIS AL
Corporate Source: ST THOMAS HOSP,DIV OBSTET & GYNAECOL,LAMBETH PALACE RD/LONDON SE1 7EH//ENGLAND/; ST GEORGE HOSP,SCH MED,MRC,EXPTL EMBRYOL & TERATOL UNIT/LONDON//ENGLAND/
Journal: IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL, 1992, V028A, N11-1 (NOV-DEC), P773-778
ISSN: 0883-8364
Language: ENGLISH Document Type: ARTICLE
Abstract: Cultured mouse embryonic stem (ES) cells are used for both in vitro and in vivo studies. The uncommitted pluripotent cells provide a model system with which to study cellular differentiation and development; they can also be used as vectors to carry specific mutations into the mouse genome by homologous recombination. To ensure successful integration into the germ line, competent totipotent diploid ES cell lines are selected using a cell injection bioassay that is both time consuming and technically demanding. The prolonged in vitro culture of rapidly dividing ES cells can lead to accumulated changes and chromosomal abnormalities that will compromise the biological function and abrogate germ line transmission of chimeric mice carrying novel genetic mutations. Such in vitro conditions will vary between individual laboratories; for example, differences in the serums used for maintenance. Using a number of different criteria we attempt in this paper to define,the parameters that we found to be key factors for optimization of the biological potential of established ES cell lines. The successful integration into the germ line is dependant on acquiring or deriving a competent totipotent mouse ES diploid cell line. In this paper parameters and criteria are defined which we found to be key factors for the optimization of the biological potential of established ES cell lines.

23/3,AB/59 (Item 2 from file: 351)
DIALOG(R)File 351:DERWENT WPI

009785822 WPI Acc No: 94-065675/08
XRAM Acc No: C94-029515
Maintaining animal embryonic stem cells in vitro without differentiation - comprises culturing the cells in presence of a feeder layer containing chicken embryonic fibroblasts
Duplicate
Patent Assignee: (CANC-) CANCER RES CAMPAIGN TECHNOLOGY; (CSIR) COMMONWEALTH SCI & IND RES ORG
Author (Inventor): DYER S L; HEATH J K; JENNINGS P; LOCKETT T
Patent Family:

CC Number	Kind	Date	Week	
WO 9403585	A1	940217	9408	(Basic)
AU 9345529	A	940303	9426	

Priority Data (CC No Date): AU 923935 (920804)
Applications (CC,No,Date): AU 9345529 (930804); WO 93AU399 (930804)
Abstract (Basic): WO 9403585 A
Maintaining in vitro animal embryonic stem (ES) cells (I) without substantial differentiation comprises culturing (I) in the presence of a feeder layer (II) comprising chicken embryonic fibroblasts (III).
Also claimed are: (A) a method as above except that (I) are

cultured in the presence of chicken derived ES cell factor; (B) an isolated chicken-derived ES stem cell factor (II) comprising a protein of mol.wt. of 20,000-30,000 (by gel filtration chromatography), an amino acid sequence in the N-terminal region of Xaa1ProValAlaGlyTyrXaa2 (Xaa1=4 unknown N-terminal amino acids, Xaa2=the remaining amino acids of the polypeptide), is derived from (III) and from chick embryo extracts and is capable of maintaining ES cells in vitro without substantial differentiation; and (C) a non-human animal derived from ES cells cultured in the presence of a feeder layer comprising (III).

USE/ADVANTAGE - Using the method it is possible to maintain in culture human or animal primordial germ cells; haemopoietic stem cells without substantial differentiation using (III) on avian factor opt. in association with other cytokine factors. ES cells offer advantages over other methods of producing transgenic animals since they are capable of in vitro genetic manipulation such as targeted mutagenesis by selective inactivation or replacement of endogenous genes and/or the introduction of genes or genetic sequence encoding new traits. Dwg.0/10

23/3,AB/60 (Item 1 from file: 352)
DIALOG(R)File 352:DERWENT WPI

010857566 WPI Acc No: 96-354519/35
XRAM Acc No: C96-111747

Purified primate embryonic stem cells capable of long term culture - for producing transgenic primates as models of human disease, and for prepn. of tissue transplants

Patent Assignee: (WISC) WISCONSIN ALUMNI RES FOUND
Author (Inventor): THOMSON J A
Patent Family:

CC Number	Kind	Date	Week	
WO 9622362	A1	960725	9635	(Basic)
AU 9647584	A	960807	9646	

Priority Data (CC No Date): US 376327 (950120)
Applications (CC,No,Date): AU 9647584 (960119); WO 96US596 (960119)
Abstract (Basic): WO 9622362 A

Purified prepn. of primate embryonic stem cells (ESC) has the following properties: (a) can proliferate in vitro for over a year; (b) maintain the normal karyotype, and retain ability to differentiate to derivs. of endoderm, mesoderm and ectoderm, throughout long-term culture; and (c) will not differentiate on a fibroblast feeder layer.

USE - The cells are used to generate transgenic primates as models of specific human genetic diseases where the gene responsible has been cloned, and in tissue transplants by adjusting culture conditions to generate specific cell types (blood, neurological or muscle cells), or by allowing the cells to differentiate in tumours. The differentiated cells can also be isolated, and transplanted to treat haematopoietic, endocrine or degenerative neurological disease or hair loss, e.g. Parkinson's disease, juvenile onset diabetes or AIDS.

ADVANTAGE - The cells resemble human cells, and can be kept in the undifferentiated state, while remaining euploid, for long periods.

Dwg.0/6

23/3,AB/61 (Item 2 from file: 352)
DIALOG(R)File 352:DERWENT WPI

009785822 WPI Acc No: 94-065675/08
XRAM Acc No: C94-029515
Maintaining animal embryonic stem cells in vitro without differentiation - comprises culturing the cells in presence of a feeder layer containing chicken embryonic fibroblasts
Patent Assignee: (CANC-) CANCER RES CAMPAIGN TECHNOLOGY; (CSIR) COMMONWEALTH SCI & IND RES ORG
Author (Inventor): DYER S L; HEATH J K; JENNINGS P; LOCKETT T
Patent Family:

CC Number	Kind	Date	Week	
WO 9403585	A1	940217	9408	(Basic)
AU 9345529	A	940303	9426	

Priority Data (CC No Date): AU 923935 (920804)
Applications (CC,No,Date): AU 9345529 (930804); WO 93AU399 (930804)
Abstract (Basic): WO 9403585 A

Maintaining in vitro animal embryonic stem (ES) cells (I) without substantial differentiation comprises culturing (I) in the presence of a feeder layer (II) comprising chicken embryonic fibroblasts (III).

Also claimed are: (A) a method as above except that (I) are cultured in the presence of chicken derived ES cell factor; (B) an isolated chicken-derived ES stem cell factor (II) comprising a protein of mol.wt. of 20,000-30,000 (by gel filtration chromatography), an amino acid sequence in the N-terminal region of Xaa1ProValAlaGlyTyrXaa2 (Xaa1=4 unknown N-terminal amino acids, Xaa2=the remaining amino acids of the polypeptide), is derived from (III) and from chick embryo extracts and is capable of maintaining ES cells in vitro without substantial differentiation; and (C) a non-human animal derived from ES cells cultured in the presence of a feeder layer comprising (III).

USE/ADVANTAGE - Using the method it is possible to maintain in culture human or animal primordial germ cells; haemopoietic stem cells without substantial differentiation using (III) on avian factor opt. in association with other cytokine factors. ES cells offer advantages over other methods of producing transgenic animals since they are capable of in vitro genetic manipulation such as targeted mutagenesis by selective inactivation or replacement of endogenous genes and/or the introduction of genes or genetic sequence encoding new traits. Dwg.0/10

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12280429 Genuine Article#: KZ595 Number of References: 1050

Title: BIOLOGICAL ROLES OF OLIGOSACCHARIDES - ALL OF THE THEORIES ARE CORRECT

Author(s): VARKI A

Corporate Source: UNIV CALIF SAN DIEGO,CTR CANC,GLYCOBIOL PROGRAM,0063,9500 GILMAN DR/LA JOLLA//CA/92093; UNIV CALIF SAN DIEGO,DIV CELLULAR & MOLEC MED/LA JOLLA//CA/92093

Journal: GLYCOBIOLOGY, 1993, V3, N2 (APR), P97-130

ISSN: 0959-6658

Language: ENGLISH Document Type: REVIEW

Abstract: Many different theories have been advanced concerning the biological roles of the oligosaccharide units of individual classes of glycoconjugates. Analysis of the evidence indicates that while all of these theories are correct, exceptions to each can also be found. The biological roles of oligosaccharides appear to span the spectrum from those that are trivial, to those that are crucial for the development, growth, function or survival of an organism. Some general principles emerge. First, it is difficult to predict a priori the functions a given oligosaccharide on a given glycoconjugate might be mediating, or their relative importance to the organism. Second, the same oligosaccharide sequence may mediate different functions at different locations within the same organism, or at different times in its ontogeny or life cycle. Third, the more specific and crucial biological roles of oligosaccharides are often mediated by unusual oligosaccharide sequences, unusual presentations of common terminal sequences, or by further modifications of the sugars themselves. However, such oligosaccharide sequences are also more likely to be targets for recognition by pathogenic toxins and microorganisms. As such, they are subject to more intra- and inter-species variation because of ongoing host-pathogen interactions during evolution. In the final analysis, the only common features of the varied functions of oligosaccharides are that they either mediate 'specific recognition' events or that they provide 'modulation' of biological processes. In so doing, they generate much of the functional diversity required for the development and differentiation of complex organisms, and for their interactions with other organisms in the environment.

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Set	Items	Description
S1	120719	STEM(W)CELL?
S2	9692	EMBRYONIC(W)S1
S3	2116211	CULTURE
S4	431009	FIBROBLAST?
S5	362	S2 AND S3 AND S4
S6	65547	STEM(W)CELL
S7	1820	EMBRYONIC(W)S6
S8	2116211	CULTURE
S9	431009	FIBROBLAST?
S10	68479	PRIMATE
S11	17948196	HUMAN
S12	281071	MONKEY OR SIMIAN
S13	5	S5 AND S10
S14	117	S5 AND S11
S15	9	S5 AND S12
S16	121	S13 OR S14 OR S15
S17	101	RD (unique items)
S18	13	NORMAL(W)KAROTYPE
S19	3801	NORMAL(W)KARYOTYPE
S20	3813	S18 OR S19
S21	4	S17 AND S20
S22	1013275	DIFFERENTIAT?
S23	61	S17 AND S22
S24	35948	ENDODERM OR MESODERM OR ECTODERM
S25	19	S23 AND S24
S26	630288	SPONTANEOUS?
S27	883	S26(W)S22
S28	1	S17 AND S27
S29	64840	CHORIONIC(W)GONADOTROPIN
S30	3	S17 AND S29
S31	73	SSEA(W)4
S32	180	SSEA(W)3
S33	195	S31 OR S32
S34	2	S17 AND S33

?t s34/3,ab/1-2

>>>No matching display code(s) found in file(s): 12, 43, 129-130, 140, 158, 187, 189, 376, 428-429, 446, 449, 452, 455-456, 636

34/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13192364 BIOSIS Number: 99192364
Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts
Thomson J A; Kalishman J; Golos T G; Durning M; Harris C P; Hearn J P
Wisconsin Regional Primate Research Center, Univ. Wisconsin, 1223 Capitol Court, Madison, WI 53715-1299, USA
Biology of Reproduction 55 (2). 1996. 254-259.
Full Journal Title: Biology of Reproduction
ISSN: 0006-3363
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 008 Ref. 124371
We report the derivation of eight pluripotent cell lines from common

marmoset (Callithrix jacchus) blastocysts. These cell lines are positive for a series of markers (alkaline phosphatase, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81) that characterize undifferentiated human embryonal carcinoma cells and rhesus embryonic stem cells. All eight cell lines had a modal chromosome number of 46; seven cell lines were XX and one was XY. Two cell lines (Cj11 and Cj62) were cultured continuously for over a year and remained undifferentiated and euploid. In the absence of fibroblast feeder layers, these cell lines differentiated to multiple cell types, even in the presence of leukemia inhibiting factor. Differentiated cells secreted bioactive CG into the culture medium and expressed alpha-CG, beta-CG, and alpha-fetoprotein mRNA, indicating trophoblast and endoderm differentiation. Bioactive CG secretion in differentiating cells was increased substantially in the presence of GnRH agoniSt D-Trp-6-Pro-9-NHEt. When grown at high densities, these cells formed embryoid bodies with a close resemblance to early postimplantation embryos, including the formation of a yolk sac, amnion, and an embryonic disc with an early primitive streak. These results make these pluripotent cells strong candidates for marmoset embryonic stem cells.

34/3,AB/2 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

201162 DBA Accession No.: 96-11933 PATENT
Purified primate embryonic stem cells capable of long term culture - for e.g. primate transgenic animal production, or tissue transplantation
AUTHOR: Thomson J A
CORPORATE SOURCE: Madison, WI, USA.
PATENT ASSIGNEE: Wisconsin-Alumni-Res.Found. 1996
PATENT NUMBER: WO 9622362 PATENT DATE: 960725 WPI ACCESSION NO.: 96-354519 (9635)
PRIORITY APPLIC. NO.: US 376327 APPLIC. DATE: 950120
NATIONAL APPLIC. NO.: WO 96US596 APPLIC. DATE: 960119
LANGUAGE: English
ABSTRACT: A new purified primate embryonic stem cell (ESC) preparation is capable of proliferation in vitro for over 1 yr, maintains a normal karyotype in prolonged culture, maintains the potential to differentiate into derivatives of endoderm, mesoderm and ectoderm (e.g. when injected into a SCID mouse), and does not differentiate when cultured on a fibroblast feeder cell layer. The stem cells spontaneously differentiate into trophoblasts, and produce chorionic gonadotropin at high cell density. The cells are SSEA-1 negative, SSEA-3 positive, SSEA-4 positive, express alkaline phosphatase (EC-3.1.3.1), are pluripotent, have normal karyotype, and may also be TRA-1-60 and TRA-1-81 positive. The cells remain euploid for over 1 yr. The ESC line is isolated by isolating blastocysts, plating inner cell mass cells on embryonic fibroblasts, dissociating the mass, re-plating on embryonic feeder cells, selecting colonies with compact morphology, and selecting and culturing cells with high nucleus to cytoplasm ratio and prominent nucleolus. The cells are used to generate transgenic primate models of genetic disease, or in tissue transplantation. (50pp)
?

36/3/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08407238 95372375
Isolation of a primate embryonic stem cell line.
Thomson JA; Kalishman J; Golos TG; Durning M; Harris CP; Becker RA; Hearn JP
Wisconsin Regional Primate Research Center, University of Wisconsin, Madison 53715-1299, USA.
Proc Natl Acad Sci U S A (UNITED STATES) Aug 15 1995, 92 (17) p7844-8, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: RR-00167, RR, NCRR; HD26458, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE

36/3/2 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13192364 BIOSIS Number: 99192364
Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts
Thomson J A; Kalishman J; Golos T G; Durning M; Harris C P; Hearn J P
Wisconsin Regional Primate Research Center, Univ. Wisconsin, 1223 Capitol Court, Madison, WI 53715-1299, USA
Biology of Reproduction 55 (2). 1996. 254-259.
Full Journal Title: Biology of Reproduction
ISSN: 0006-3363
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 008 Ref. 124371

36/3/3 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

8599092 EMBASE No: 92275000
Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture
Matsui Y.; Zsebo K.; Hogan B.L.M.
Department of Cell Biology, Tuberculosis/Cancer Research Inst., Sendai 980 Japan
CELL (USA) , 1992, 70/5 (841-847) CODEN: CELLB ISSN: 0092-8674
LANGUAGES: English SUMMARY LANGUAGES: English

36/3/4 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

201162 DBA Accession No.: 96-11933 PATENT
Purified primate embryonic stem cells capable of long term culture - for e.g. primate transgenic animal production, or tissue transplantation
AUTHOR: Thomson J A

CORPORATE SOURCE: Madison, WI, USA.
PATENT ASSIGNEE: Wisconsin-Alumni-Res.Found. 1996
PATENT NUMBER: WO 9622362 PATENT DATE: 960725 WPI ACCESSION NO.: 96-354519 (9635)
PRIORITY APPLIC. NO.: US 376327 APPLIC. DATE: 950120
NATIONAL APPLIC. NO.: WO 96US596 APPLIC. DATE: 960119
LANGUAGE: English

36/3/5 (Item 1 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

14131596 Genuine Article#: RR817 No. References: 63
Title: IN-VITRO PLURIPOTENCY OF EPIBLASTS DERIVED FROM BOVINE BLASTOCYSTS
Author(s): TALBOT NC; POWELL AM; REXROAD CE
Corporate Source: USDA ARS,BELTSVILLE AGR RES CTR,INST LIVESTOCK & POULTRY SCI,GENE EVALUAT & MAPPING LAB/BELTSVILLE//MD/20705; USDA ARS,BELTSVILLE AGR RES CTR,INST LIVESTOCK & POULTRY SCI,GENE EVALUAT & MAPPING LAB/BELTSVILLE//MD/20705
Journal: MOLECULAR REPRODUCTION AND DEVELOPMENT, 1995, V42, N1 (SEP), P 35-52
ISSN: 1040-452X
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

36/3/6 (Item 2 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13790200 Genuine Article#: QQ271 No. References: 75
Title: ISOLATION AND CHARACTERIZATION OF PERMANENT CELL-LINES FROM INNER CELL MASS CELLS OF BOVINE BLASTOCYSTS
Author(s): VANSTEKELENBURGHAMERS AEP; VANACHTERBERG TAE; REBEL HG; FLECHON JE; CAMPBELL KHS; WEIMA SM; MUMMERY CL
Corporate Source: NETHERLANDS INST DEV BIOL,HUBRECHT LAB,UPPSALALAAN 8/3584 CT UTRECHT//NETHERLANDS/; NETHERLANDS INST DEV BIOL,HUBRECHT LAB/3584 CT UTRECHT//NETHERLANDS/; INRA/JOUY EN JOSAS//FRANCE/; AFRC,ROSLIN INST/ROSLIN/MIDLOTHIAN/SCOTLAND/; FREE UNIV AMSTERDAM HOSP,IVF LAB/AMSTERDAM//NETHERLANDS/
Journal: MOLECULAR REPRODUCTION AND DEVELOPMENT, 1995, V40, N4 (APR), P 444-454
ISSN: 1040-452X
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

36/3/7 (Item 3 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13513739 Genuine Article#: PU209 No. References: 37
Title: ISOLATION AND CULTURE OF INNER CELL MASS CELLS FROM HUMAN BLASTOCYSTS
Author(s): BONGSO A; FONG CY; NG SC; RATNAM S
Corporate Source: NATL UNIV SINGAPORE HOSP,DEPT OBSTET & GYNAECOL,LOWER KENT RIDGE RD/SINGAPORE 0511//SINGAPORE/
Journal: HUMAN REPRODUCTION, 1994, V9, N11 (NOV), P2110-2117
ISSN: 0268-1161

Language: ENGLISH   Document Type: ARTICLE   (Abstract Available)
?

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S37          57   TRA(W)1(W)60
S38          23   TRA(W)1(W)81
S39          57   S37 OR S38
?t s17 and s39

>>>'AND' not allowed in command
?s s17 and s39

             101  S17
              57  S39
     S40       3  S17 AND S39
?t s40/6/1-3


 40/6/1     (Item 1 from file: 155)
08407238   95372375
  Isolation of a primate embryonic stem cell line.


 40/6/2     (Item 1 from file: 5)
13192364     BIOSIS Number: 99192364
  Pluripotent cell lines derived from common marmoset (Callithrix jacchus)
blastocysts
  Print Number: Biological Abstracts Vol. 102 Iss. 008 Ref. 124371


 40/6/3     (Item 1 from file: 357)
201162  DBA Accession No.: 96-11933
Purified primate embryonic stem cells capable of long term culture - for
    e.g. primate transgenic animal production, or tissue transplantation
?
```

Set	Items	Description
S1	120719	STEM(W)CELL?
S2	9692	EMBRYONIC(W)S1
S3	2116211	CULTURE
S4	431009	FIBROBLAST?
S5	362	S2 AND S3 AND S4
S6	65547	STEM(W)CELL
S7	1820	EMBRYONIC(W)S6
S8	2116211	CULTURE
S9	431009	FIBROBLAST?
S10	68479	PRIMATE
S11	17948196	HUMAN
S12	281071	MONKEY OR SIMIAN
S13	5	S5 AND S10
S14	117	S5 AND S11
S15	9	S5 AND S12
S16	121	S13 OR S14 OR S15
S17	101	RD (unique items)
S18	13	NORMAL(W)KAROTYPE
S19	3801	NORMAL(W)KARYOTYPE
S20	3813	S18 OR S19
S21	4	S17 AND S20
S22	1013275	DIFFERENTIAT?
S23	61	S17 AND S22
S24	35948	ENDODERM OR MESODERM OR ECTODERM
S25	19	S23 AND S24
S26	630288	SPONTANEOUS?
S27	883	S26(W)S22
S28	1	S17 AND S27
S29	64840	CHORIONIC(W)GONADOTROPIN
S30	3	S17 AND S29
S31	73	SSEA(W)4
S32	180	SSEA(W)3
S33	195	S31 OR S32
S34	2	S17 AND S33
S35	156575	ALKALINE(W)PHOSPHATASE
S36	8	S17 AND S35
S37	57	TRA(W)1(W)60
S38	23	TRA(W)1(W)81
S39	57	S37 OR S38
S40	3	S17 AND S39
S41	46270	TROPHOBLAST?
S42	10	S17 AND S41

?t s42/3,ab/7

>>>No matching display code(s) found in file(s): 12, 43, 129-130, 140, 158, 187, 189, 376, 428-429, 446, 449, 452, 455-456, 636

42/3,AB/7   (Item 2 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13513739   Genuine Article#: PU209   Number of References: 37
Title: ISOLATION AND CULTURE OF INNER CELL MASS CELLS FROM HUMAN BLASTOCYSTS
Author(s): BONGSO A; FONG CY; NG SC; RATNAM S
Corporate Source: NATL UNIV SINGAPORE HOSP,DEPT OBSTET & GYNAECOL,LOWER

KENT RIDGE RD/SINGAPORE 0511//SINGAPORE/
Journal: HUMAN REPRODUCTION, 1994, V9, N11 (NOV), P2110-2117
ISSN: 0268-1161
Language: ENGLISH Document Type: ARTICLE
Abstract: Totipotent non-committed inner cell mass (ICM) cells from human blastocysts, if demonstrated to be capable of proliferating in vitro without differentiation, will have several beneficial uses, not only in the treatment of neurodegenerative and genetic disorders, but also as a model in studying the events involved in embryogenesis and genomic manipulation. Nine patients admitted to an in-vitro fertilization programme donated 21 spare embryos for this study. All 21 embryos were grown from the 2-pronuclear until blastocyst stages on a human tubal epithelial monolayer in commercial Earle's medium (Medicult, Denmark) supplemented with 10% human serum. The medium was changed after blastocyst formation to Chang's medium supplemented with 1000 units/ml of human leukaemia inhibitory factor (HLIF) and the embryos left undisturbed for 72 h to allow the hatched ICM and trophoblast to attach to the feeder monolayer. Nineteen of the 21 embryos from nine patients produced healthy ICM lumps which could be separated and grown in vitro. Two of the lumps differentiated into fibroblasts while the remaining 17 (eight patients) produced cells with typical stem cell-like morphology, were alkaline phosphatase positive and could be maintained for two passages. It was possible to retain the stem cell-like morphology, alkaline phosphatase positiveness and normal karyotype through the two passages in all of them using repeated doses of HLIF every 48 to 72 h. This is the first report on the successful isolation of human ICM cells and their continued culture for at least two passages in vitro.

?

=> d l14 kwic 1-4

US PAT NO: 5,532,156 [IMAGE AVAILABLE] L14: 1 of 4

ABSTRACT:
Continuous . . . epiblasts of pig blastocysts. The cultures are feeder-dependent and grow slowly with doubling times of 3 to 4 days. They **differentiate** into large secretory duct-like structures or form small canaliculi. Alternatively, the cells accumulate droplets that stain intensely with oil red O, a lipid-specific stain. .alpha.-Fetoprotein and albumin mRNA expression increases as the cells **differentiate** in culture.

SUMMARY:

BSUM(3)

This . . . self-renewing cell population. The stem cell characteristics of the hepatocytes indicate that the cells are unique for investigations of liver **differentiation** and organogenesis.

SUMMARY:

BSUM(7)

It . . . supra). A consensus of data indicated that liver stem cells would express AFP and albumin and would be capable of **differentiating** into bile duct cells and parenchymal hepatocytes (Shiojiri, N., J. Embryol. Exp. Morphol., 79: 149-152, 1981; Evarts et al. Cancer. . .

DRAWING DESC:

DRWD(7)

FIG. 4A-4D is photomicrographs showing secondary cultures of pig epiblast colonies. a, secondary neuroectodermal-like pig epiblast colony with neural rosettes and **differentiated** neuronlike cells (scale bar, 26 um); b, pigment accumulation in neuroectodermal-like secondary colony (scale bar, 15 um); c, PICM-9 contracting. . .

DRAWING DESC:

DRWD(8)

FIG. 5A-5E is photomicrographs showing **differentiated** cells of secondary pig epiblast cell cultures. a,b, PICM-19 after 2 wk in maintenance co-culture stained with oil red O. . .

DRAWING DESC:

DRWD(11)

FIG. 8A-8D is photomicrographs showing the hepatocyte-like morphology and **differentiation** of PICM cells in STO co-culture. a, PICM-35, Passage 7, oil red O stain after 3 wk in static culture with **differentiation** into open lumen ductal structures and intracellular and extracellular accumulations of lipid (scale bar, 88 um); b, PICM-19,

Passage 39,. . .

DETDESC:

DETD(3)

A . . . cells of the blastocyst. First, the culture of the intact pig blastocyst results in the attachment and growth of the **trophoblast** and primitive endoderm, but under these conditions, unlike the mouse blastocyst, the pig epiblast usually does not grow but instead degenerates. In addition, it has been reported (Mummery et al., **Differentiation**, 46: 51-60, 1991) that visceral endoderm can be a potent inducer of mesodermal **differentiation** for mouse embryonal carcinoma (EC) cells. Thus, the prevention of interaction between the pig epiblast cells and trophectodermal or endodermal. . .

DETDESC:

DETD(7)

Primary culture of the isolated pig epiblasts on STO feeder cells produced homogeneous colonies whose cells resembled mouse embryonic stem (**ES**) **cells** (small round cells with large nuclei and nucleoli, FIG. 2). Pig epiblast cells were approximately the same size as the cells of the D3 mouse **ES** **cell** line. Like mouse **ES** **cells**, the cells grew as tight clusters either on top of or nested in between the STO feeder cells. Also, the. . .

DETDESC:

DETD(8)

After . . . (3 of 14), what seemed to be a clonal expansion of an epithelial cell type began within the colonies. These **differentiated** cells were flat, cuboidal and translucent, with large nuclei but small nucleoli. They rapidly produced a cell sheet which pushed. . . was characteristic of the ICM endoderm, sometimes appeared as a component of the complex colony type. These cells may have **differentiated** from the epiblast. However, they also may have derived from endoderm cells that were accidentally carried over during the physical. . .

DETDESC:

DETD(9)

Secondary . . . grew very slowly after passage and soon became senescent. Several of the neuronlike epiblast colonies also gave rise to terminally **differentiated** pigment-producing cells if the cells had clumped during early passage (FIG. 4b and FIG. 5e). For example, one of the. . . entirely as grossly clumped groups of cells at passage 2. The majority of PICM-9 clumps failed to grow and instead **differentiated** into pigmented cells. In addition, after 2 to 3 weeks in maintenance culture, at least four other **differentiated** phenotypes devloped from the PICM-9 subculture (Table 1). Among these were small fibroblastic cells, neuronlike cells, and one colony which. . .

DETDESC:

DETD(10)

Six . . . two cell types with prominent nuclei and nucleoli. The first of these, represented by PICM-16, were very similar to mouse **ES** **cells** in appearance (FIG. 6a). PICM-16 grew on top of the STO feeder layer as numerous individual clusters of cells after. . . confluency. Despite treatment with retinoic acid or DMSO, these epithelial-like cultures (PICM-16 and 17) have not shown any other specialized **differentiation** potential besides formation of polarized epithelium.

DETDESC:

DETD(12)

The . . . red O stain. The vacuole formation and oil red O staining were not seen in those cells that had undergone **differentiation** into the glandlike tubules (FIG. 5a,b).

DETDESC:

DETD(18)

Added human recombinant leukemia inhibitory factor (LIF) at 10 or 20 ng/ml or purified erythroid **differentiation** factor (EDF) at 50 to 100 ng/ml had no apparent effect on the morphology, maintenance of morphology, or growth of. . . STO feeder cells. In contrast, LIF at the same concentration maintained the morphology and growth potential of the D3 mouse **ES** **cells** when passaged in the absence of STO cells.

DETDESC:

DETD(20)

The . . . W., Vertebrate fetal membranes. New Brunswick, N.J.: Rutgers University Press, 1987:74-78; Solter and Knowles, PNAS, 72:5099-5102, 1975). Inasmuch as the **trophoblast** cell layer surrounding the ICM is only one-cell thick, it is probable that no **trophoblast** cells survive the immunosurgery. This was found to be the case by Solter and Knowles (1975, supra) in their original. . . strong attachment. This arrangement suggests that the endoderm cells migrated out from the epiblast core as they surrounded it and **differentiated**.

DETDESC:

DETD(22)

TABLE 1

CULTURING PIG EPIBLAST CELLS
PIG EPIBLAST CELL CULTURE DERIVATIONS AND CHARACTERISTICS

Primary Colony Morphology	Demonstrated **Differentiation** Potential	Cell Morphology	Cell Culture Designation	PDP.sup.a on STO	PDP.sup.a off STO	Karyotype

feeder
feeder

Complex (25%). . .

DETDESC:

DETD(23)

Although . . . embryonic development exist between mice and ungulates (Mossman, 1987, supra), under similar culture conditions the morphology of their embryonic stem (**ES**) **cells** should be very similar, given the following: a) the **ES** **cells** are derived from the ICM at a point before embryonic **differentiation**; b) they share the same functional attribute of totipotency, and structure often follows function; and c) hamster **ES** **cells** and human EC cells are very similar in morphology to mouse **ES** **cells** (Andrews et al., I. Cell lines from human germ cell tumours. In: Robertson, E. J., ed. Teratocarcinomas and **embryonic** **stem** **cells**: a practical approach. Oxford: IRL Press, 1987: 207-248; and Doetschman et al., J. Embryol. Exp. Morphol., 87: 27-45, 1985). This. . . this morphology was difficult, however, because as with mouse epiblasts (Hogan et al., 1977, supra), the pig epiblast cells quickly **differentiated**.

DETDESC:

DETD(24)

Mouse **ES** **cells** often suffer extensive cell death or **differentiation** if passaged in the absence of feeder cells (Robertson, E. Embryo-derived stem cell lines. In: Robertson, E., ed. Teratocarcinomas and **embryonic** **stem** **cells**: a practical approach. Oxford: IRL Press, 1987: 71-112). The cytokine LIF, also known as **differentiation** inhibitory activity (DIA), is the factor secreted from STO feeder cells which maintains mouse **ES** **cells** in an undifferentiated state (Rathjen et al., Cell, 62:1105-1114, 1990; Smith and Hooper, Exp. Cell. Res., 145: 458-462, 1983; and Williams et al., Nature, 336: 684-687, 1988). EDF (Activin-A) has also been found to suppress the **differentiation** of mouse EC cells (van den Eijnden-van Raaij et al., A. Mech. Dev., 33: 157-166, 1991). Neither of these cytokines had a **differentiation**-inhibiting effect on the pig epiblast cultures. This was the case at both the primary colony stage and with the various. . . pig epiblast cells did not respond to LIF in the same manner as mouse epiblast cells. This apparent lack of anti-**differentiation** or proliferation response to added recombinant LIF has also been observed in certain human EC cell lines (Pera at al., **Differentiation**, 42: 10-23, 1989; P. Andrews, personal communication, 1992) and in the culture of pig and sheep ICM-derived cells (Piedrahita et. . . of effect but this was not unexpected because it also failed to maintain the undifferentiated phenotype of the D3 mouse **ES** **cell** line when grown without STO feeder cells.

DETDESC:

DETD(25)

The . . . with very gentle methods, such as lipid-buffered

trypsin-EDTA, the cells are easily killed. A critical requirement reported for establishing mouse **ES** **cell** lines is the early dispersion and subculturing of the primary epiblast growths (Robertson, 1987, supra). This presumably short-circuits cell-to-cell interactions which lead to **differentiation** induction. The initial dispersions of the pig epiblast colonies usually resulted in a significant amount of cell clumping, and, analogous to mouse ES and EC cell lines, this clumping may have stimulated **differentiation** in spite of the presence of LIF.

DETDESC:

DETD(26)

The pig epiblast cells of the primary and secondary cultures spontaneously **differentiated** into various cell types, which seem to be mesodermal, neuroectodermal, ectodermal, and endodermal in character. As with some mouse EC cell lines, the final **differentiation** events of the pig epiblast cultures occurred after several weeks in maintenance culture on STO feeder cells (Nicolas et al., Cancer Res., 36: 4224-4231, 1976). It has long been recognized that the **differentiation** of various cells is induced by paracrine regulatory interactions or cell-to-cell interactions with mesenchymal cells (Balinsky, B. I., An introduction. . . 566-578; Drew, A. H., Br. J. Exp. Pathol., 4: 46-52, 1923; Gorbstein, C., Exp. Cell. Res., 13: 575-587, 1957). The **differentiation** of the pig epiblast cells may be driven by the STO feeder cells elaborating combinations of growth factors which do not predispose to prevent **differentiation**. For example, it has been shown that cultured fibroblasts secrete hepatocyte growth factor to the extent that it induces epithelial. . . et al., Cell, 67: 901-908, 1991). Also, among the known pleiotropic effects of LIF is its ability to promote the **differentiation** of neural crest cells and hemopoietic cells, to stimulate myoblast proliferation, and to activate hepatocytes (Hilton and Gough, J. Cell.. . . Biochem., 46:21-26, 1991; Mummery, 1991, supra). Given this, it is very possible that co-culture on STO cells contributed to the **differentiation** observed in the pig epiblast cultures.

DETDESC:

DETD(27)

The . . . no gross changes in PICM-16's karyotype has occurred after two successive single cell clonings. Thus, PICM-16 is similar to mouse **ES** **cell** lines in being immortal and in retaining a normal male karyotype (Robertson and Bradley. Production of permanent cell lines from. . .

DETDESC:

DETD(28)

Senescence . . . cultivation of some cell types and, in general, is thought to be necessary for the isolation and maintenance of mouse **ES** **cells** (Robertson, 1987, supra; Tookey, J. I., PNAS, 72: 73-77, 1975). In addition, mouse embryonic fibroblasts have recently been shown to. . .

DETDESC:

DETD(29)

The **differentiation** of the pig epiblast cell cultures was brought about by cell aggregation combined with long-term culture. Those cell cultures (PICM-8,. . . tests indicate that PICM-8 displays a mitogenic response to LIF and EDF, both of which are known to support the **differentiation** and maintenance of neuronal cells (Murphy et al., PNAS, 88: 3498-3501, 1991: Schubert et al., Nature, 344: 868-870, 1990). The. . . it is likely that this degree of pluripotency is lost with continued passage of the cells. The cell cultures that **differentiated** into glandlike structures or adipocytelike cells (PICM-13, 18, 19) had a distinctive morphology which is typical of parenchymal hepatocytes, thus. . .

DETDESC:

DETD(30)

The cell line, PICM-16, which is epithelial in character and most closely resembles mouse **ES** **cells**, has occasionally formed domes. Although mouse **ES** **cell** lines are not known to form domes in culture, a human EC cell line, NEC 14, apparently shares this characteristic with PICM-16 (Haseyawa et al., **Differentiation**, 47: 107-117, 1991). PICM-16 is morphologically similar to the ICM-derived pig cells isolated by other groups. Only limited information has. . .

DETDESC:

DETD(46)

The . . . and PICM-19 also showed no evidence of polyploidy and confirmed the porcine origin of the cells. All the cell cultures **differentiated** to multi-cellular duct-like structures and into colony groupings that either accumulated and secreted lipid or developed spaces between individual cells similar to biliary canaliculi (FIG. 8a,b,c). The ductal and adipogenic **differentiation** phenotypes appeared to be mutually exclusive and occurred spontaneously over the course of 1 to 3 weeks in static co-culture. . .

DETDESC:

DETD(47)

It . . . can be seen at the top of FIG. 8c, individual canaliculi coalesced to form longer networks of interconnected canaliculi. Final **differentiation** into a mature ductal structure appeared to occur from these interconnected canaliculi. Duct formation was a terminal **differentiation** event since the constituent cells would no longer divide and grow if passaged, although they remained viable. Similarly, cultures which **differentiated** to adipogenesis grew very slowly when passaged and could not be sustainably cultured. In contrast, colonial areas that developed small canaliculi could be cloned and passaged to produce mass cultures without loss of **differentiation** potential. Thus, the development of canaliculi was non-terminal **differentiation** which could be a precursor to the terminal ductal or adipogenic **differentiation** programs.

DETDESC:

DETD(48)

Phase-contrast microscopy of the fully **differentiated** duct-like structures showed phase-dense material at the lumenal surface of the structures (FIG. 8a). This arrangement indicates a secretory structure. A modified form of the duct-like **differentiation** structure occurred in the PICM-19 culture after about 50 population doublings. This formation was characterized by the absence of a. . .

DETDESC:

DETD(49)

Primary . . . and Weber, Cell, 31: 303-306, 1982; Marceau et al., Can. J. Biochem. Cell Biol., 63: 448-457, 1985; Levy et al. **Differentiation**, 39: 185-195, 1988).

DETDESC:

DETD(53)

It . . . reached a peak in PICM-19 cultures that had remained in static culture for 2 to 3 weeks and had extensively **differentiated** into ductal structures (FIG. 9, lanes 4 and 9). A relatively high expression was maintained in culture for an additional. . .

DETDESC:

DETD(62)

In . . . single-cell cloning of PICM-19 was performed at least six times with the establishment of mass cultures of the subclones. The **differentiation** potential of the cells did not change over the passage history of the PICM-19 culture. The subclones of PICM-19 also. . .

DETDESC:

DETD(63)

The . . . an environment suitable for the isolation and maintenance of hepatocarcinoma cells (Aden et al., Nature (London), 282: 615-616, 1979), mouse **embryonic** **stem** **cells** (Robertson, 1987, supra) and mouse primordial germ cells (Matsui et al., Cell, 70: 841-847, 1992; Resnick et al., Nature, 359:. . .

DETDESC:

DETD(64)

The . . . is the only known example of a sustainable culture of pluripotent parenchymal hepatocytes. Their fetal phenotype and their ability to **differentiate** into ductal structures, adipogenic cells, and monolayers of larger polygonal cells indicate that the PICM cells represent early hepatocyte progenitors. . . hepatocytes from the

26-day pig fetal liver. These cell cultures formed monolayers of polygonal cells with interconnective canaliculi that could **differentiate** further to produce extracellular lipid or multicellular ductal structures. However, preliminary results indicate that the fetal liver-derived hepatocyte cultures are. . . and senesce after variable degrees in in vitro passage. Thus, epiblast culture enables a derivation of fetal hepatocytes prior to **differentiation** into committed progenitor cells.

ABSTRACT:
Transgenic swine, and compositions and methods for making and using same, are provided. Central to the invention are porcine (Sus scrofa) **embryonic** **stem** **cell** lines and methods for establishing them. Cells of such lines are transformed with exogenous genetic material of interest and then. . .

SUMMARY:

BSUM(2)

This invention relates to compositions and methods for making swine **embryonic** **stem** **cells**, chimeric swine from the stem cells, and transgenic swine from the chimeras.

SUMMARY:

BSUM(5)

One of the methods to generate transgenic animals, the use of transformed **embryonic** **stem** **cells** (**ES**-**cells**), has shown certain advantages over other methods when used to produce mouse chimeras, from which transgenic mice are derived. Once isolated, **ES**-**cells** may be grown in vitro for many generations producing unlimited numbers of identical cells capable of developing into fully formed. . .

SUMMARY:

BSUM(6)

A second major advantage of **ES**-**cells** is that they can be genetically manipulated in vitro. **ES**-**cells** may be transformed by introducing exogenous DNA into the cells via electroporation. Following transformation individual **ES**-**cell** clones may be screened in vitro for the incorporation of the exogenous DNA before being used to produce chimeric embryos (Thomas et al., 1987). **ES**-**cell** clones containing the transferred DNA can be selected and used for blastocyst injection. The ability to screen and select transformed **ES**-**cells** in vitro is one of the most important features for utilizing this strategy to produce transgenic animals.

SUMMARY:

BSUM(7)

When transformed **ES**-**cells** are used to make chimeric embryos, some of these cells may be incorporated into the gonads and participate in the. . .

SUMMARY:

BSUM(9)

The . . . providing the transgene, with endogenous DNA. Although the majority of such recombinational events are non-homologous reactions, many cell types (including **ES**-**cells**) also possess the enzymatic machinery required for homologous recombination. The homology-dependent recombination between exogenous DNA and chromosomal DNA sequences is. . . of genetic alterations/mutations created in vitro to precise sites within the host cellular genome. If the host cells are pluripotent **ES**-**cells**, such alterations can then be transferred to the germ line of a living organism.

SUMMARY:

BSUM(10)

ES **cells** have been used to produce transgenic lines of mice which through homologous recombination have directed gene insertion. This strategy of. . . changes has immense potential in agriculture, and in furthering our understanding of the genetic control of mammalian development. However, the **ES**-**cell** method has not been successfully applied to production of larger transgenic mammals, for example, transgenic swine. A reason for the. . .

SUMMARY:

BSUM(12)

Notarianni . . . (1990) report methods to produce transgenic pigs by use of pluripotent stem cells but do not convincingly show that pluripotent **embryonic** **stem** **cells** were produced. Chimeric pigs were not reported as an intermediate step toward production of a transgenic pig. Pluripotent cells are. . .

SUMMARY:

BSUM(13)

The . . . Evans patent application, International Publication No. WO90/03432, and publications from his group are more reminiscent of epithelial cells, than of **embryonic** **stem** **cells** from other organisms such as the mouse. Indeed, the authors state the "**ES**" **cells** from pigs are morphologically dissimilar from mouse **ES**-**cells**. Also, no biochemical tests were done to confirm that the selected cells were not **differentiated**. The only evidence of pluripotency was production of **differentiated** cells in culture.

SUMMARY:

BSUM(14)

Even if some **embryonic** **stem** **cells** were actually mixed into the "selected" cell population reported by Evans, use of these cell populations to produce chimeric pigs would be expected to be relatively inefficient because chance would dictate whether an **embryonic** **stem** **cell** would be included in the injected material. The probability of inclusion would be expected to be proportional to the percentage of **embryonic** **stem** **cells** in the mixed culture. The lack of a homogeneous culture would lead to inefficient and unpredictable results. Moreover, the method disclosed could not be described as "a

method to produce **embryonic** **stem** **cells**," which implies homogeneity and reproducibility.

SUMMARY:

BSUM(17)

Handyside (1987) attempted to produce chimeric sheep from **embryonic** **stem** **cells**, but was admittedly unsuccessful. Flake (1986) produced chimeras, but resorted to in utero transplant. Doetschman (1988) identified "**embryonic** **stem** **cells**" from hamsters by growing them on mouse embryonic fibroblast feeder layers. Pluripotency was determined by **differentiation** in suspension cultures. Ware (1988) reported embryo derived cells from "farm animals" growing on Buffalo Rat Liver BRL and mouse. . .

SUMMARY:

BSUM(21)

Embryonic **stem** **cell** transfer to produce transgenic pigs is an improvement over available methods. A reason that **embryonic** **stem** **cell**-mediated gene transfer has not been employed in domestic livestock is the lack of established, stable **embryonic** **stem** **cell** lines available from these species. The availability of such lines would provide feasible methods to produce transgenic animals.

SUMMARY:

BSUM(22)

Previous failures to identify and isolate **ES** **cells** in swine, may have been due in part to the expectation that such cells would be fast-growing and resemble those. . .

SUMMARY:

BSUM(24)

In the present invention, limitations of the art are overcome by the production of stable, pluripotent swine **embryonic** **stem** **cell** cultures. These cell cultures are used to make chimeric pigs, an intermediate step in producing a transgenic pig. The invention. . .

SUMMARY:

BSUM(26)

The . . . art of producing transgenic swine by presenting a novel and reproducible method which includes use of stable, swine embryonic stem (**ES**) **cell** lines as host vehicles for gene transfer into swine. Transgenic animals possess an alteration in their DNA which has been. . .

SUMMARY:

BSUM(27)

In the present method, a cell from an **embryonic** **stem** **cell** line is introduced into a host embryo to create a chimeric pig, a certain percentage of which pigs so formed. . . offspring of the breeding that exhibit transgenic expression. These are sometimes referred to as "germline transgenics." Alternatively, nuclei from the **ES** **cell** line are transferred into a cell from which an embryo develops.

SUMMARY:

BSUM(28)

Among the advantages of using **embryonic** **stem** **cells** to produce transgenic swine, are that efficiency is improved, and that transformed **ES**-**cells** can be used as the progenitors of clonal lines (descendent lines having the same genotype as the single parental cell,. . .

SUMMARY:

BSUM(29)

A . . . the reproducible incorporation of a nucleotide sequence into a specific location of the host genome. Many cell types, including the **embryonic** **stem** **cells** of the present invention, possess the enzymatic machinery necessary to direct homologous recombination.

SUMMARY:

BSUM(30)

In . . . incorporated into a specific site of a host cell genome. If the transformed host cell is a pluripotent or totipotent **embryonic** **stem** **cell**, and said stem cell is incorporated into a chimeric pig, a transgenic animal is produced with a specific genetic change. . . stem cell is defined as an undifferentiated cell which is capable of being induced to develop into more than one **differentiated** cell type; a totipotent stem cell is defined as an undifferentiated cell which is capable of being induced to develop. . . The requirement for proceeding from a chimeric to a transgenic pig, is that a gamete is a descendant of an **embryonic** **stem** **cell**. Existence of stable cell cultures allows development of a clone of **ES** **cells** with the same altered genetic complement, therefore, the opportunity arises to make replica swine with the same genetic complement.

SUMMARY:

BSUM(33)

Versatility in the kinds of genetic manipulation possible in **embryonic** **stem** **cell** cultures, reproducibility of the methods to make such cultures, and predictability of results of genetic manipulation are other advantageous aspects. . .

SUMMARY:

BSUM(34)

A method for producing a chimeric swine includes an initial step of

introducing a swine embryonic stem (**ES**) **cell** which preferably is totipotent and that has a first genetic complement, into a host embryo which has a second swine. . .

SUMMARY:

BSUM(39)

Swine are generally of the genus and species Sus scrofa. In an illustrative embodiment, the chimera comprises **embryonic** **stem** **cells** from a first breed of swine, for example, the Meishan line and a morula from a second breed of swine,. . .

SUMMARY:

BSUM(55)

An initial step in the method is to establish a stable, undifferentiated embryonic stem (**ES**) **cell** line. For purposes of the present invention, stable means maintaining essentially similar cell types and growth parameters, through serial subcultures,. . . maintaining a stable chromosome complement of about 38. Undifferentiated in this context means not showing morphological or biochemical evidence of **differentiation**. An **embryonic** **stem** **cell** is an undifferentiated cell which is capable of **differentiating** into embryonic structures. An **embryonic** **stem** **cell** line is derived from a culture of **embryonic** **stem** **cells**. Using methods disclosed herein, **ES**-**cells** were developed from Meishan, Yorkshire and Duroc swine. Efficiency of producing **ES**-**cells** is somewhat affected by strain or breed of donor. Other suitable breeds or types include the NIH mini-pigs, feral pigs,. . .

SUMMARY:

BSUM(56)

A preliminary step in isolating swine **embryonic** **stem** **cells** is to collect swine embryos. Female pigs are checked for estrus, preferably twice daily. Donor sows for the **ES**-**cells** are inseminated at the time of the female pig's estrus. Embryos are then collected on days 5.5-7.5 post estrus if. . .

SUMMARY:

BSUM(57)

Embryo . . . culture dishes, temperature, and other conditions. In an illustrative embodiment, embryos are grown on or with feeder layers of cells. **Differentiated** cells will not attach to the feeder layer, or attach poorly. After about 24-48 hours in culture, expanded blastocysts generally. . .

SUMMARY:

BSUM(58)

Initial . . . blastocyst (HB) plates down and attaches with the inner

cell mass (ICM) growing up like a hilus or polyp. The **trophoblast** cells grow outward from the ICM, leaving a clear zone between the ICM and the **trophoblast** cells. This configuration allows for easy plucking of the ICM, essentially free of **trophoblast** cell contamination. The isolated ICM can then be put in trypsin to dissociate the cells for further subculture.

SUMMARY:

BSUM(59)

On . . . a large clump and then begins to spread out as if it were melting. Consequently, the ICM is associated with **trophoblast** cells and its configuration resembles a fried egg in appearance. This phenomenon makes it difficult initially (first several days, 1-5). . . plucked or the entire plated embryo may be trypsinized to dissociate the cells. After discrete multilayered clumps or colonies of **ES** **cells** are visible then plucking is done to isolate these cells from contaminating **trophoblast** and/or other **differentiated** cell types. This results in purification of cells with the proper ES morphology.

SUMMARY:

BSUM(60)

Embryonic **stem** **cells** are isolated from the attached embryos and maintained in cultures. The inner cell mass (ICM) of the cultured embryo is. . .

SUMMARY:

BSUM(62)

Serial . . . support growth. Subculturing the culture is continued until a stable culture with morphological features and growth parameters characteristic of an **embryonic** **stem** **cell** culture is established.

SUMMARY:

BSUM(63)

As a preliminary scan for pluripotency of the **ES** **cell** lines, undifferentiated morphology is sought using the light microscope. Morphologically **ES**-**cells** are small (about 8-15 microns in diameter) and rounded, and possess large dark nuclei which contain one or more prominent. . .

SUMMARY:

BSUM(64)
(a) introducing a first **embryonic** **stem** **cell** from a culture into an immunodeficient mammal;

SUMMARY:

BSUM(65)
(b) allowing a tumor to form in the mammal from the **embryonic**

stem **cell**;

SUMMARY:

BSUM(67)
(d) selecting a second **embryonic** **stem** **cell** from the tumor culture.

SUMMARY:

BSUM(68)

Lack of **differentiation** may also be determined by absence of cytoskeletal structural proteins such as cytokeratin 18 and vimentin, which are only expressed in **differentiated** cell types. Conversely, ability of the cells to **differentiate** after induction, is detected by loss of typical undifferentiated **ES**-**cell** morphology and positive fluorescent antibody staining with anti-cytokeratin 18 and anti-vimentin.

SUMMARY:

BSUM(69)

Established **embryonic** **stem** **cells** grow rapidly, dividing about every 18-36 hours. To protect against spontaneous, unwanted **differentiation**, cells are generally kept at a high density. Changing media and subculturing are used to maintain healthy, cultures of the. . .

SUMMARY:

BSUM(70)

Transformation of an **embryonic** **stem** **cell** in vitro with a first genetic complement which includes a nucleotide sequence is accomplished by any of the methods known. . .

SUMMARY:

BSUM(71)

After . . . desired stage, generally the morula or blastocyst stage. The morula stage is preferred because the cells are fewer and less **differentiated** than cells of the blastocyst, consequently, a higher percentage of chimerism in more diverse cell types, is expected. Other stages. . .

SUMMARY:

BSUM(72)

Any . . . chimeric pig is produced. The chimerism is detected by an assay for the gene that was introduced via the transformed **embryonic** **stem** **cell**. For example, a skin pigment gene not present in the host blastocyst genome, may be detected as spots in the. . .

SUMMARY:

BSUM(75)

To produce a transgenic pig, the genetic complement, for example, an isolated nucleotide sequence initially used to transform an **embryonic** **stem** **cell** of the present invention, must be incorporated into the genome of the host. If the transforming nucleotide sequence consists of. . .

SUMMARY:

BSUM(88)

ES **cells** introduced into SCID (or other immune deficient or immuno-comprimised mice) mice produce tumors. These may be teratomas or teratocarcinomas, comprised of a number of fully **differentiated** tissues (including: muscle, bone, fat, cartilage, skin, epithelia, nervous, glandular, hemapoetic, secretory and the like). Each line of transgene carrying **ES** **cells** can be injected into SCID (or other immune deficient or immuno-comprimised mice) and the tumors harvested. In situ hybridization, immunocytochemistry,. . .

DRAWING DESC:

DRWD(2)

FIGS. 1A-1D are a comparison of morphological characteristics of development of cells designated "stem cells" by Evans (top panel) and the "**embryonic** **stem** **cells** of the present invention" (bottom panel).

DRAWING DESC:

DRWD(4)

(FIG. 1B) cluster nest of undifferentiated "**embryonic** **stem** **cells**" from an established cell line of the present invention at 200.times. magnification;

DRAWING DESC:

DRWD(6)

(FIG. 1D) multilayered growth of the "**embryonic** **stem** **cells**" from an established cell line of the present invention at 200.times. magnification.

DRAWING DESC:

DRWD(7)

FIG. 2 **ES** **cells** of the present invention stained wth Giemsa at 400.times.; cells are dispersed and fixed on slides.

DETDESC:

DETD(3)
1. Purification of Undifferentiated Embryonic Stem (**ES**) **Cell** Lines

DETDESC:

DETD(7)

After . . . onto plates containing only conditioned medium (treatment 1), or plated onto STO feeder layers (treatment 2), as disclosed herein. PROTOCOL: **ES** **cell** colonies are dislodged from the underlying cells and washed through two changes of calcium/magnesium-free PBS. Alternatively, the entire dish of. . . a total of approximately 20% fetal calf serum (FCS), .beta.-mercaptoethanol, antibiotics, nucleosides and non-essential amino acids (Smith and Hooper, 1987). **ES** **cells** in both treatments are allowed to grow in the culture.

DETDESC:

DETD(9)

After . . . colonies are either plucked (treatment 1) or the whole dish (treatment 2) is placed onto plates containing only conditioned medium. **ES** **cells** in both treatments are allowed to grow in culture. Feeder layers may also be used to support growth, but is. . .

DETDESC:

DETD(12)

After . . . colonies are either plucked (treatment 1) or the whole dish (treatment 2) is placed onto plates containing only conditioned medium. **ES** **cells** in both treatments are allowed to grow in culture.

DETDESC:

DETD(17)

The . . . cultured in only CSCM (or with feeder cells through step 2) are passed every 2-4 days in only CSCM. Until **ES** **cell** lines with consistent morphology, size 8-15.mu., with a nuclear to cytoplasmic ratio of .about.85:15, and growth characteristics (doubling time 18-36 h) are established. This entire process (Steps 1-6) may take from 5-21 weeks to isolate a single **ES** **cell** line. There lines are then used for production of chimeras and/or nuclear transfer. The next step is required to identify. . .

DETDESC:

DETD(19)

This step in the isolation procedure involves injection of the **ES**-**cells** underneath the tunica albuginea of the testis of immune system compromised mice (SCID, irradiated nude) to produce teratocarcinomas. The mice. . . for the presence of tumors daily. When palpable tumors are observed the mouse is euthanized and the tumor harvested. Undifferentiated **ES** **cells** are recovered from the tumor and re-introduced into in vitro culture. **ES** **cell** lines with appropriate morphology, size 8-15.mu., with a nuclear to cytoplasmic ratio of .about.85:15, and growth characteristics (doubling time of. .

DETDESC:

DETD(20)
NOTE: This step may occur at any point where **ES** **cells** of proper morphology are observed.

DETDESC:

DETD(22)

Periodically it is necessary to pluck colonies as outlined above and re-isolate the **ES** **cells** with consistent morphology, size 8-15.mu., with a nuclear to cytoplasmic ratio of .about.85:15, and growth characteristics (doubling time of 18-36. . .

DETDESC:

DETD(23)
NOTE: Maintenance of these isolated, purified undifferentiated **ES** **cell** lines is required to insure the proper cell type for generation of chimeras and for nuclear transfer. Some **differentiation** occurs spontaneously during in vitro culture and as a result of the freezing process. These **differentiated** cells do not subculture well, but occasionally it is necessary to re-purify the **ES** **cells** from the **differentiated** cells.

DETDESC:

DETD(25)

To obtain enriched populations of **ES** **cells** (size 8-15.mu. with a nuclear to cytoplasmic ratio of .about.85:15, and doubling time of 18-36 h) for chimera production or nuclear transfer, **ES** **cell** colonies were dislodged from the underlying cells and washed through two changes of calcium/magnesium-free PBS. The colonies were then transferred. . .

DETDESC:

DETD(26)

Purification of swine **ES**-**cells** may also be performed by centrifugal elutriation, flow cytometry, unit gravity sedimentation, differential centrifugation, cell separation, immuno-surgery to preferentially kill mouse cells or **differentiated** swine cells, plucking of colonies or individual cells, differential or immuno-staining, production of chimeric embryos and re-isolation of inner cell. . .

DETDESC:

DETD(28)

ES **cell** Colonies are dislodged from the underlying cells and washed through two changes of calcium/magnesium-free PBS. The colonies are then transferred. . .

DETDESC:

DETD(30)

Plates containing **ES** **cell** colonies and underlying cells are washed through two changes of calcium/magnesium-free PBS. The plates had 1-5 ml of trypsin solution. . .

DETDESC:

DETD(31)

TABLE 1

COMPARISON OF METHODS OF MAKING **EMBRYONIC** **STEM** **CELL** LINES

	Mice	Evans	Wheeler
Embryo Stage	3.5 d blast.	6.5-11 d blasts..sup.a	6.5-10 d hatched. . .

DETDESC:

DETD(32)
2. In Vitro Characterization of **ES**-**Cell** Lines

DETDESC:

DETD(33)

An aspect of the invention is to select a transformed **embryonic** **stem** **cell** in vitro which is likely to produce a chimeric state when introduced into a pig embryo. The selection criteria are based on morphological characteristics of the transformed **embryonic** **stem** **cell**. Generally, morphological characteristics identifiable by inspection of the cell using the light microscope are predictive, although other assays for predictive. . .

DETDESC:

DETD(34)

Swine **embryonic** **stem** **cells** of the present invention are translucent, epithelial-like in appearance, and tend to form colonies or nests (clumps) of multilayers as. . . morphology. The doubling rate of these cells is about 18-36 hours. These characteristics differ little from those reported for mouse **embryonic** **stem** **cells**, but do differ significantly from those reported by Evans. (FIG. 1).

DETDESC:

DETD(35)

Similarities of swine to mouse **embryonic** **stem** **cells** include that the nucleus to cytoplasmic ratio is approximately 85:15. The nucleus is round and contains several prominent nucleoli. Cell. . .

DETDESC:

DETD(36)

In . . . and those of the present invention are set forth. Also, the similarities between the swine cells disclosed herein and the **ES** **cells** of mice are described.

DETDESC:

DETD(37)

TABLE 2

COMPARISON OF CELL MORPHOLOGY OF MICE AND OF SWINE **ES** **CELLS**

Parameter	Mice	Evans Swine	Swine of the Present Invention
Size	11-12 .mu.m	"larger	8-15 .mu.m

. . .

DETDESC:

DETD(38)
3. Teratoma/Teratocarcinoma Assay for Swine **Embryonic** **Stem** **Cells**

DETDESC:

DETD(39)

It . . . teratoma/teratocarcinomas when introduced into syngeneic host mice. Therefore, this test was incorporated into the screening process during development of porcine **ES** **cell** lines. A teratoma is a true neoplasm composed of bizarre and chaotically arranged tissues that are foreign embryologically, as well. . .

DETDESC:

DETD(40)

All lines which are truly pluripotent should proliferate, **differentiate** and form tumors in severe combined immunodeficient mice (SCID) or other immunologically noncompetent animals. Those cell lines which produced tumors. . .

DETDESC:

DETD(41)

The . . . animals were euthanized and examined for the presence of tumors. Cells from the tumor were then put into the porcine **ES** **cell** culture system. During 7 days of culture, some cells **differentiated** while others maintained their original embryonic

undifferentiated morphology. These undifferentiated colonies were then selected, isolated and grown up for use. . .

DETDESC:

DETD(42)
4. In Vitro **Differentiation** of Pluripotent **ES**-**Cells**

DETDESC:

DETD(43)

True **ES**-**cells** are induced to **differentiate** in vitro into ectoderm, mesoderm, and endoderm. There is a concomitant loss during said **differentiation** of characteristics of undifferentiated **ES**-**cell** morphology as described herein for swine, and elsewhere for the mouse.

DETDESC:

DETD(44)

A method for inducing **differentiation** in **ES**-**Cells** is to culture cell lines such as D49/6-E and M144-B at high density on feeder layers until the cells form. . .

DETDESC:

DETD(46)

The culture media is changed about every 48 hours and cells are examined daily for evidence of **differentiation**. Generally, about 30-40% of the cells terminally **differentiate** under these conditions, that is, reach a recognizable cell type according to criteria known to those of skill in the. . .

DETDESC:

DETD(47)

Complex . . . similar to structures reported in mice. Less commonly, neuronal-like cells also are found in these cultures. The nature of the **differentiated** cell types is determined by immunofluorescence as described in the methods section herein.

DETDESC:

DETD(48)

Undifferentiated, pluripotent cells lack the cytoskeletal structural proteins cytokeratin 18 and vimentin, which are only expressed in **differentiated** cell types. Antibodies are available which are directed against antigenic structures which are indicative of cellular **differentiation**. (Rudnicki and McBurney, 1987). Examples of these structures include neurofilaments (expressed in ectoderm), glial fibrillar protein (expressed in ectoderm), keratin (expressed in endoderm) and desmin (expressed in mesoderm). Formation of antigen-antibody complexes are indicative of a **differentiated** state;

conversely, absence of an antigen-antibody reaction is evidence for lack of **differentiation**.

DETDESC:

DETD(49)

Evidence of pluripotency is provided by **differentiation** of structures from all the embryonic layers, from a single cell line.

DETDESC:

DETD(50)

Pluripotent cells lack the cytoskeletal structural proteins, cytokeratin 18 and vimentin, which are only expressed in **differentiated** cell types. Positive staining against specific antigens, including neurofilaments (expressed in ectoderm), glial fibrillar acidic protein (expressed in ectoderm), keratin (expressed in endoderm) and desmin (expressed in mesoderm), is indicative of cellular **differentiation**. Replicate colonies of ES-like cells exhibiting undifferentiated morphology were examined for the presence or absence of staining for vimentin, cytokeratin. . .

DETDESC:

DETD(51)

. . .

- - -

NF 68,160,200.sup.4
+ - + -

.sup.1 Letters in parentheses indicate treatment,
T = **differentiation** induced,
C = untreated control lines,
STO = embryonic fibroblast controls were negative for all antibodies tested;. . .

DETDESC:

DETD(52)
5. In Vivo **Differentiation** of Pluripotent **ES**-**Cells**

DETDESC:

DETD(53)

In vivo **differentiation** of pluripotent **ES**-**cells** was tested by determining the ability of the cells to produce chimeras. To produce chimeras, about 10-20 potentially **ES**-**cells** were injected into the blastocoele of 6-7 day old swine embryos. This procedure is similar to that described for production. . .

DETDESC:

DETD(54)

In an illustrative embodiment, Meishan swine **ES**-**cells** (MW/M175F) were injected into Duroc embryos. Duroc swine are characterized as having red hair and pink skin pigmentation. Meishan swine. . . black hair and black pigment appear against a red-brown background if a chimera is produced. In the converse embodiment, Duroc **ES**-**cells** are injected into Meishan embryos, and red-brown hair and spots would appear on a black hair, black skin background, if. . .

DETDESC:

DETD(55)

TABLE 4

PRODUCTION OF PORCINE CHIMERAS
BY MICROINJECTION OF MEISHAN **EMBRYONIC**
STEM **CELLS** TO DUROC RECIPIENT EMBRYOS

Recipient Breed	No. Embryos Transferred	No. Live Born Piglets	No. Coat Color. . .

DETDESC:

DETD(58)
6. Uses for **Embryonic** **Stem** **Cells**

DETDESC:

DETD(59)

a) . . . by the host organism of these transplanted materials may be produced. Exogenous foreign or homologous DNA is transferred to porcine **ES** **cells** by electroporation, exposure to calcium phosphate, microinjection, lipofection, retro- or other viral or microbial vector or other means. The **ES** **cells** are screened for incorporation for this DNA or expression of antigens, directly transferred to embryos to produce chimeras, or used. . .

DETDESC:

DETD(60)

Production of **differentiated** cells for replacement, repair or augmentation of damaged, non-functional, or impaired cells or tissues are another use. Exogenous foreign or homologous DNA are transferred to porcine **ES** **cells** by electroporation, calcium phosphate, microinjection, lipofection, retro- or other viral or microbial vector or other means. The **ES** **cells** are screened for incorporation for this DNA, directly transferred to embryos to produce chimeras, or used in nuclear transfer systems. . .

DETDESC:

DETD(61)

b. . . . biological molecules--pharmaceuticals, diagnostics, antibodies, used in manufacturing or processing, as food supplements of additives and the like, are produced using **ES** **cells**. Exogenous foreign or homologous DNA are transferred to porcine **ES** **cells** by electroporation, calcium phosphate, microinjection, lipofection, retro- or other viral or microbial vector or other means. The **ES** **cells** are screened for incorporation for this DNA, or are directly transferred to embryos to produce chimeras, or are used in. . .

DETDESC:

DETD(66)

c. Enhance genetic traits in livestock--Porcine **ES** **cells** are used to improve disease resistance; growth rate and efficiency; milk production, quality and composition; carcass quality and composition; body. . . against pathogens, increased secretion of growth promotants, stimulation of reproductive processes including lactation is contemplated. Genetically-engineered individuals resulting from porcine **ES** **cells** serve as founder animals for new breeds or strains of swine. For example, altered milk protein composition allows for increased. . .

DETDESC:

DETD(67)

Removing . . . Specific DNA sequences are removed, introduced or altered to manipulate the biology of the individual. Genetically-engineered individuals resulting from porcine **ES** **cells** serve as foundation animals for new breeds or strains of swine. For example, removing the gene encoding the enzyme responsible. . .

DETDESC:

DETD(68)

d. Production of "genetically engineered" identical offspring is accomplished by the transfer of **ES** **cell** nuclei to embryonic cells or unfertilized oocytes, such that resultant cell lines, tissues, organs or offspring contain all or part. . .

DETDESC:

DETD(69)

ES **cells** from specific cell lines, either with or without an exogenous gene or genes, are transferred by micromanipulation to foreign cytoplasm. . . and/or organs or transferred to surrogate mothers for production of genetically engineered offspring. Transfer of multiple cells or a single **ES** **cell** or nucleus to an enucleated oocyte or embryonic cell is accomplished through micromanipulation. Fusion of the transferred cell or nucleus. . . glycol, or by exposure to ionophores that alter the ionic fluxes of the cell membranes. Genetically-engineered individuals resulting from porcine **ES** **cells** serve as foundation animals for new breeds or strains of swine. For example; **ES** **cells** carrying a transgene may be fused to enucleated oocytes to produce cells with identical nuclear DNA for production of cloned. . .

DETDESC:

DETD(73)

The production of human clotting factor IX (FIX) in the milk of transgenic swine via **embryonic** **stem** **cells** is accomplished by the following protocol. The human clotting factor IX protein encoding sequence is excised from the FIX cDNA. . .

DETDESC:

DETD(79)

ES **cell** culture medium (SCM) consisted of Dulbecco's modified Eagle's medium (DMEM; containing L-glutamine, 4500 mg glucose/L) with 0.1 mM 2-mercaptoethanol, 50. . .

DETDESC:

DETD(81)

5. Stem cell isolation and culture: **Embryonic** **stem** **cells** were isolated from the attached embryos and maintained in culture by the following protocol. The inner cell mass (ICM) enlarges. . .

DETDESC:

DETD(82)

The . . . growth rate. Cells had spent media replaced with fresh media every 2-3 days. To preliminarily characterize the pluripotent nature of **ES**-**cell** lines we used microscopic observation of undifferentiated morphology. **ES**-**cells** are typically small and rounded, possessing large dark nuclei which contain one or more prominent nucleoli.

DETDESC:

DETD(83)

ES **cells** were purified, as described herein, from feeder cells or from **differentiated** porcine cells (lines were developed entirely in conditioned medium (CSCM) alone). Further characterization requires indirect immunofluorescent staining of **ES**-**cells** for lack of the cytoskeletal structural proteins, cytokeratin 18 and vimentin, which are only expressed in **differentiated** cell types. In vitro **differentiation** of pluripotent **ES**-**cells** into endoderm, ectoderm or mesodermwith concomitant loss of typical undifferentiated **ES**-**cell** morphology and positive staining with anti-cytokeratin 18 and anti-vimentin antibodies may be induced.

DETDESC:

DETD(84)

6. Culture of **embryonic** **stem** **cells**: Once established, stem

cells grow rapidly, dividing every 18-36 hours. The cells should be kept at relatively high densities to ensure that a high rate of cell division is maintained as this minimizes the level of spontaneous **differentiation**. The cultures were re-fed daily, or according to the acidity of the medium, and subcultured at 3-4 day intervals. Cells. . .

DETDESC:

DETD(86)

7. Production of chimeras: After stable **ES**-**cell** lines are established, they are used to produce chimeric swine embryos. This is to test the ability of the cell. . . or blastocysts are recovered, as described above, and placed in 100 .mu.l of PBS under oil. The embryos have 5-50 **ES**-**cells** placed into the blastocoele cavity by means of a glass injection needle attached to a micromanipulator. After injection, the embryos. . .

DETDESC:

DETD(87)

8. Production of Chimeras and Clones via Nuclear Transfer: Chimeras are produced by aggregation of **ES** **cells** with pre-implantation embryos of the following stages: one-cell, two-cell, four-cell, eight-cell, 16-cell, 32-cell, morula, blastocyst, and hatched blastocyst. Chimeras are also produced by injection of **ES** **cells** with pre-implantation embryos of the following stages: one-cell, two-cell, four-cell, eight cell, 16-cell, 32-cell, morula, blastocyst, and hatched blastocyst.

DETDESC:

DETD(88)

Nuclear transfer offspring or clones are produced by fusion or injection of **ES** **cells** with enucleated, preimplantation embryonic cells of the following stages of embryo: oocytes, one-cell, two-cell, four-cell, eight-cell, 16-cell, 32-cell, morula, blastocyst,. . .

DETDESC:

DETD(89)

In vivo **differentiation** of pluripotent **ES**-**cells** was confirmed by their ability to participate in the formation of chimeric offspring. Morula, blastocyst and expanded blastocyst stage embryos. . . a fine glass holding pipette attached to a micromanipulator (Narashige Inc., Tokyo, Japan). Five to 20, range one to 30, **ES**-**cells** were placed into the cell mass (morula) or into the blastocoele cavity (blastocyst and expanded blastocyst) by means of a. . .

DETDESC:

DETD(90)

Chimeras . . . (i.e., Meishan and Duroc). Chimeric embryos were produced using two coat color markers: Meishan (black hair with black skin pigmentation) **ES**-**cells** were injected into Duroc (red-brown

hair with pink skin pigmentation) embryos. These combinations allowed for easy visual detection of chimeric. . .

DETDESC:

DETD(101)

TABLE 11

MOUSE **ES** **CELL** CULTURE MEDIUM FOR THE ISOLATION AND MAINTENANCE OF MURINE **ES** **CELLS** IN VITRO

Ingredient	mM	gm/L	Volume (ml)
DMEM (Table 6)	--	--	80.0
Fetal bovine serum	--	--. . .	

Filter sterilized, stored at 4.degree. C., and used within 2 weeks. Warm to 37.degree. C. before use with **ES** **cells**.
This medium allows the isolation and proliferation of embryonal cell line
from mouse blastocysts when in coculture with mitoticallyinhibited. . .

DETDESC:

DETD(105)

11. Immunofluorescence as a Measure of **Differentiation** in Pluripotent Porcine **Embryonic** **Stem** **Cells**

DETDESC:

DETD(107)
ES **cell** lines to test several fetal pigs

DETDESC:

DETD(164)
Axelrod, H. R., 1984, **Embryonic** **stem** **cell** lines derived from blastocysts by a simplified technique, Dev. Biol. 101:225-228.

DETDESC:

DETD(166)
Doetschman et al., 1988, Establishment of hamster. blastocyst-derived embryonic stem (**ES**) **cells**, Dev. Biol. 127:224-227.

DETDESC:

DETD(170)
Gossler, A., Doetschman, T., Korn, R., Serfling, E., Kemler R. 1986. Transgenesis by means of blastocyst-derived **embryonic** **stem** **cell** lines. Proc. Natl. Acad. Sci. USA 83:9065-9069.

DETDESC:

DETD(175)

Notarianni et al., Maintenance and **differentiation** in culture of pluripotential embryonic cell lines from pig blastocysts, J. Reprod. Fert. Suppl. 41 1990, 51-56.

DETDESC:

DETD(176)
Piedrahita, J. A., Anderson, G. B., BonDurrant, R. H. 1990b. On the isolation of **embryonic** **stem** **cells**: Comparative behavior of murine, porcine and ovine embryos. Therio. 34:879-901.

DETDESC:

DETD(180)
Robertson, E. J., 1987, Embryo-derived stem cell lines, in Teratocarcinomas and **embryonic** **stem** **cells**: a practical approach, Robertson (ed.), pp. 71-112. IRL Press, Ltd., Oxford, England.

DETDESC:

DETD(182)
Rudnicki, M. A. and McBurney, M. W., Cell culture methods and induction of **differentiation** of approach, (E. J. Robertson, Ed.), pp. 19-49. IRL Press Limited, Oxford, England.

DETDESC:

DETD(183)
Smith, A. G. and M. L. Hooper. 1987, Buffalo rat liver cells produce a diffusible activity which inhibits the **differentiation** of murine embryonal carcinomas and **embryonic** **stem** **cells**. Dev. Biol. 121:1.

DETDESC:

DETD(184)
Strojek, R. M. et al., 1990, A method for cultivating morphologically undifferented **embryonic** **stem** **cells** from porcine blastocysts, Herrogenology 33:901-913.

CLAIMS:

CLMS(1)

What is claimed is:

1. A method of obtaining an **embryonic** **stem** **cell** for incorporation into a swine embryo to form a chimeric swine, said method comprising:
(a) introducing a cell from a. . .
a feeder layer, and
(ii) subculturing the culture until a stable culture with morphological features and growth parameters characteristic of an **embryonic** **stem** **cell** culture is established, into a SCID mouse;
(b) allowing a tumor to form in the mouse from the cell; and
(c) obtaining an **embryonic** **stem** **cell** from a culture that is shown to be capable of producing a tumor in step b.

CLAIMS:

CLMS(2)

2. The method of claim 1, wherein the **embryonic** **stem** **cell** is characterized by an undifferentiated morphology indistinguishable from the morphology of a cell from the culture of step a of. . .

CLAIMS:

CLMS(3)

3. . . . method for determining the cell types in which a genetic complement is expressed, said method comprising:
(a) introducing a swine **embryonic** **stem** **cell** Which comprises the genetic complement into an immunocompromised mouse to produce a tumor;
(b) placing the tumor in suitable conditions to allow the tumor to **differentiate** into a plurality of recognizable cell types and to empress the genetic complement;
(c) excising the tumor; and
(d) analyzing the **differentiated** cell types to determine in which cell types the genetic complement is expressed.

CLAIMS:

CLMS(4)

4. An **embryonic** **stem** **cell** obtained from a culture that is capable of forming a tumor in a SCIDS mouse in accordance with the method. . .

CLAIMS:

CLMS(5)

5. A culture initiated from an **embryonic** **stem** **cell** of claim 4.

US PAT NO: 5,366,888 [IMAGE AVAILABLE] L14: 3 of 4

```
L1           102 S EMBRYONIC(W)STEM(W)CELL#
L2            63 S ES(W)CELL#
L3           119 S L1 OR L2
L4          1032 S CHORIONIC(W)GONADOTROPIN
L5             9 S L3 AND L4
L6          1053 S TRA
L7             0 S L3 AND L6
L8            36 S SSEA
L9             1 S L3 AND L8
L10            0 S TRA(W)1(W)60
L11            0 S TRA(W)1(W)81
L12          124 S TROPHOBLAST#
L13        54075 S DIFFERENTIAT###
L14            4 S L3 AND L12 AND L13
L15            0 S SCIS(W)MOUSE
L16           39 S SCID(W)MOUSE
L17            7 S L16 AND L3
L18        44111 S IMMUNE OR IMMUNO########## OR IMMUNITY
=> s l17 and l18
L19            5 L17 AND L18
```

```
S1      120719   STEM(W)CELL?
S2        9692   EMBRYONIC(W)S1
S3     2116211   CULTURE
S4      431009   FIBROBLAST?
S5         362   S2 AND S3 AND S4
S6       65547   STEM(W)CELL
S7        1820   EMBRYONIC(W)S6
S8     2116211   CULTURE
S9      431009   FIBROBLAST?
S10      68479   PRIMATE
S11   17948196   HUMAN
S12     281071   MONKEY OR SIMIAN
S13          5   S5 AND S10
S14        117   S5 AND S11
S15          9   S5 AND S12
S16        121   S13 OR S14 OR S15
S17        101   RD (unique items)
S18         13   NORMAL(W)KAROTYPE
S19       3801   NORMAL(W)KARYOTYPE
S20       3813   S18 OR S19
S21          4   S17 AND S20
?t s21/3,ab/1-4
```

>>>No matching display code(s) found in file(s): 12, 43, 129-130, 140, 158, 187, 189, 376, 428-429, 446, 449, 452, 455-456, 636

21/3,AB/1 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

201162 DBA Accession No.: 96-11933 PATENT
Purified primate embryonic stem cells capable of long term culture - for e.g. primate transgenic animal production, or tissue transplantation
AUTHOR: Thomson J A
CORPORATE SOURCE: Madison, WI, USA.
PATENT ASSIGNEE: Wisconsin-Alumni-Res.Found. 1996
PATENT NUMBER: WO 9622362 PATENT DATE: 960725 WPI ACCESSION NO.: 96-354519 (9635)
PRIORITY APPLIC. NO.: US 376327 APPLIC. DATE: 950120
NATIONAL APPLIC. NO.: WO 96US596 APPLIC. DATE: 960119
LANGUAGE: English
ABSTRACT: A new purified primate embryonic stem cell (ESC) preparation is capable of proliferation in vitro for over 1 yr, maintains a normal karyotype in prolonged culture, maintains the potential to differentiate into derivatives of endoderm, mesoderm and ectoderm (e.g. when injected into a SCID mouse), and does not differentiate when cultured on a fibroblast feeder cell layer. The stem cells spontaneously differentiate into trophoblasts, and produce chorionic gonadotropin at high cell density. The cells are SSEA-1 negative, SSEA-3 positive, SSEA-4 positive, express alkaline phosphatase (EC-3.1.3.1), are pluripotent, have normal karyotype, and may also be TRA-1-60 and TRA-1-81 positive. The cells remain euploid for over 1 yr. The ESC line is isolated by isolating blastocysts, plating inner cell mass cells on embryonic fibroblasts, dissociating the mass, re-plating on embryonic feeder cells, selecting colonies with compact morphology, and selecting and culturing cells with high nucleus to cytoplasm ratio and prominent nucleolus. The cells are used to generate transgenic

```
Set      Items   Description
S1           4   HUMAN(W)TERATOCARCINOMA(W)MUCIN(W)LIKE(W)ANTIGEN
S2           1   RD (unique items)
S3          58   TRA(W)1(W)60
S4          23   TRA(W)1(W)81
S5          23   S3 AND S4
S6           7   RD (unique items)
S7           4   S6 NOT PY>1995
?t s7/6/1-4
```

7/6/1 (Item 1 from file: 155)
08407238 95372375
Isolation of a primate embryonic stem cell line.

7/6/2 (Item 2 from file: 155)
07816422 93180444
Retinoic acid-induced differentiation of the developmentally pluripotent human germ cell tumor-derived cell line, NCCIT.

7/6/3 (Item 3 from file: 155)
05952011 87220949
Human embryonal carcinoma cells and their differentiation in culture.

7/6/4 (Item 4 from file: 155)
04312063 85129225
Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells.
?t s7/3,ab/4

>>>No matching display code(s) found in file(s): 12, 43, 129-130, 140, 158, 187, 189, 376, 428-429, 446, 449, 452, 455-456, 636

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04312063 85129225
Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells.
Andrews PW; Banting G; Damjanov I; Arnaud D; Avner P
Hybridoma (UNITED STATES) Winter 1984, 3 (4) p347-61, ISSN 0272-457X
Journal Code: GFS
Contract/Grant No.: CA29894, CA, NCI; CA23097, CA, NCI; CA38405, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Two monoclonal antibodies (TRA-1-60 and TRA-1-81) recognizing distinct cell surface antigens on human embryonal carcinoma (EC) cells were produced and characterized. These antibodies reacted strongly with undifferentiated human EC cells in indirect radioimmunoassays (RIA) and immunofluorescence (IF) assays, but only weakly or not at all with cells derived from